

Determination of Erythrocyte Potassium and Glutathione Polymorphism in Saanen, Maltese and Turkish Hair Goats

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Abstract: Main purpose of this study was to investigate genetic polymorphisms of Saanen (S), Maltese (M) and Turkish Hair (TH) goats based on erythrocyte potassium and glutathione types. It was also designed to detect the association between these components and some blood parameters depending on the breed comparisons. The phenotypic and alleles frequencies were calculated for potassium and glutathione types of all three breeds. The difference between low and high potassium (LK and HK, respectively) types for the means of erythrocyte potassium concentration (K_e) were statistically important for all breeds ($p < 0.01$). The significant relationships between erythrocyte potassium types and blood parameters of erythrocyte sodium (Na_e), whole blood potassium (K_{wb}), plasma potassium (K_p), erythrocyte potassium (K_e) were observed for S and TH goats. On the other hand, the important relationships were detected between erythrocyte potassium types and blood parameters of whole blood sodium (Na_{wb}), Na_e , K_{wb} , K_p , K_e , total monovalent cation concentration in erythrocyte ($Na_e + K_e$) for M goat breed ($p < 0.05$). The correlation coefficient between Na_e and K_e blood parameters was important and it was ranged from -0.26 to -0.34. In addition, Maltese goat breeds had a higher K_e than the others but Na_e was higher in TH goats. Even if parity and birth type differences were not statistically important for all blood parameters, there was indication of significant differences for these values except hematocrit value (PCV), Na_e and GSH concentrations depending on the potassium types within breed ($p < 0.05$) and also among all breed analyses ($p < 0.001$).

Key words: Erythrocyte potassium, plasma sodium, glutathione, blood polymorphism, Saanen, Maltese, Turkish hair goat

INTRODUCTION

The study was designed to investigate blood polymorphisms in Saanen, Maltese and Turkish hair goats in Turkey. Those breeds were chosen since Saanen is recently the most preferred dairy goat in Turkey due to its high milk yield and high persistency (Koyuncu and Pala, 2008). Thus, it has been heavily used in crossbreeding programs. Moreover, Maltese goats were brought to Turkey in 15th century and they have been common ever since in Turkey and they are the most adapted goats to the region (Uysal-Pala *et al.*, 2006). On the other hand, Turkish hair goat was chosen since it is the native and the most predominant goat breed in Turkey (Toplu and Altinel, 2008a, b). Many researches were conducted to study blood polymorphism in various livestock species in several countries for a long time (Khan and Taneja, 1983; De La Haba *et al.*, 1991; Tunon *et al.*, 1987;

Gonzales *et al.*, 1984; Soysal *et al.*, 2003; Gurcan *et al.*, 2010). Some studies were also carried out in this field in Turkey but there were not enough studies conducted to compare different breeds in local goat breeds. Therefore, it was needed to compare the exotic breeds with the native Turkish breeds in terms of erythrocyte potassium and glutathione polymorphisms.

Potassium and glutathione are important components in metabolic activities in mammalian species since the potassium ion is an essential in regulating osmotic balance between cells and the extracellular fluid (Ellory and Tucker, 1974; Campbell, 1987). On the other hand, Glutathione (GSH) is an intracellular tripeptide found in blood and it is a useful antioxidant, protecting the haemoglobin and the cells in general from oxidative damage (Castillo *et al.*, 2001; Lu, 2008). Therefore, since these components play an important role on metabolic activity of animal, the deficiency of these parameters

could affect negatively health and efficiency of the animals. Several studies in literature stated significant relationships between potassium, glutathione types and production traits in livestock (Tucker, 1974; Antunovic *et al.*, 2004; Milewski and Szczepanski, 2006; Gunes *et al.*, 2008; Board *et al.*, 2009). Similarly, Atroshi and Sandholm (1982) detected an important relationship between GSH and milk production traits. Therefore as the several researchers reported, these relationships could be used as indirect selection criteria to improve animal production level. Major purpose of this study was to determine the genetic structures of Saanen, Maltese and Turkish hair goats based on erythrocyte potassium and glutathione types. Also, it was aimed to display the differences among some blood parameters depending on the potassium types in erythrocytes, breeds, parity and birth type of animals.

MATERIALS AND METHODS

In this study, 25 Saanen, 51 Maltese and 49 Turkish hair goats located in Canakkale, Turkey were used. The body weights of the mature goats used in the study was approximately 25-30 kg and the lactation milk yield was 300-500 kg. All animals depended heavily on pasture and were fed roughage with 0.5 kg mixed feed during the time of the study.

Blood collection and blood parameters detection: In total, blood samples were acquired from 125 female goats. The samples were obtained using 2×10 mL sterilized and labeled vacuum tubes coated with lithium heparin, an anticoagulant from the external jugular vein. The erythrocyte potassium and glutathione polymorphisms were investigated using the blood samples. In addition, the samples were employed to discover and interpret any relationship with major blood parameters including whole blood sodium (Na_{wb}), plasma sodium (Na_p), erythrocyte sodium (Na_e), whole blood potassium (K_{wb}), plasma potassium (K_p), erythrocyte potassium (K_e) and total monovalent cation concentration in erythrocyte (Na_e+K_e). Percent Hematocrit Value (PCV) was measured using the microhematocrit method defined by Burtis and Ashwood (1994). The erythrocyte glutathione concentrations were scanned using the spectrophotometer at 412 nm. The erythrocyte glutathione concentrations (GSH) were estimated using the following formula as mg dL⁻¹ in erythrocyte:

$$GSH = [\text{Observed GSH value}/(\text{PCV} \cdot 10^{-2})]$$

Erythrocyte glutathione types were named as GSH^l (low glutathione) type for animals if the concentration of GSH^l was ≤20 mg dL⁻¹ and as GSH^h (high glutathione) for

animals if the glutathione concentration was >20 mg dL⁻¹ (Ekmekci and Mert, 2009). Erythrocyte potassium and sodium concentrations were estimated using the amount of sodium and potassium in plasma and in whole blood. In addition, hematocrit values were calculated based on the following formula as mmol L⁻¹ (Gonzales *et al.*, 1984):

$$K_e = \frac{K_p + [(K_{wb} - K_p)]}{[(\text{PCV} \cdot 10^{-2})]}$$

$$Na_e = \frac{Na_p + [(Na_{wb} - Na_p)]}{[(\text{PCV} \cdot 10^{-2})]}$$

Where:

- K_p = Concentrations of K in plasma
- Na_p = Concentrations of Na in plasma
- K_{wb} = Concentrations of K in whole blood
- Na_{wb} = Concentrations of Na in whole blood
- K_e = Concentrations of K in erythrocyte
- Na_e = Concentrations of Na in erythrocyte
- PCV = Packed Cell Volume

Erythrocyte potassium types were called LK (low potassium) for animals if the concentrations of K_e were equal to or <13 mmol L⁻¹ and HK (high potassium) for animals if the K_e concentrations were >13 mmol L as it was defined by Galip and Elmaci (2001). The allele frequencies were estimated using the square root method for potassium and glutathione types as defined by Cotterman (1954) because low potassium alleles (K^L) were dominant to high potassium alleles (K^H), conversely GSH^h were dominant over to GSH^l. Hardy-Weinberg Equilibrium was tested using the Chi-square (χ^2) test to see if the differences between the observed and expected genotypic frequencies were small enough to confirm to the equilibrium.

Statistical analysis: The analysis included descriptive statistics and correlation analysis to detect the relationships among blood parameters. The potassium types of animals were analyzed using the t-test and also the effects of breeds, parity and birth type on some blood parameters were investigated by Applying the Variance Analysis (ANOVA) and Multivariate Analysis of Variance (MANOVA) tests. The statistical analysis were carried out using SPSS (IBM SPSS Statistics 18, 2010) and SAS programs (SAS, 1999).

RESULTS AND DISCUSSION

The research findings are shown in Table 1 for blood parameters based on animal erythrocyte potassium types for all breeds. Differences between the LK and HK

Table 1: Least square means and standard errors of blood parameters for erythrocyte potassium types of Saanen, Maltese and Turkish Hair goat breeds

Blood parameters ¹	Potassium types (mean±SE)					
	Saanen breed (S)		Maltese breed (M)		Turkish Hair breed (TH)	
	LK (n = 9)	HK (n = 16)	LK (n = 14)	HK (n = 37)	LK (n = 12)	HK (n = 37)
PCV	23.2±1.40	23.4±1.00	21.6±0.8	22.4±0.6	23.0±1.2	22.9±0.50
Na _{wb}	176.6±5.20	166.2±4.50	178.7±9.6 ^a	164.6±3.7 ^b	183.9±5.4	178.5±2.60
Na _p	159.8±7.20	153.4±5.30	157.3±6.8	154.7±4.4	158.2±7.1	152.3±3.70
Na _e	239.1±6.50 ^a	210.0±4.04 ^b	214.4±7.6 ^a	190.3±9.8 ^b	275.9±6.4 ^a	236.5±3.90 ^b
K _{wb}	14.3±3.40 ^d	28.3±2.60 ^f	30.4±3.3 ^b	35.5±1.7 ^a	17.4±2.8 ^d	33.5±1.00 ^e
K _p	15.3±4.30 ^b	27.7±2.50 ^a	30.5±4.3 ^b	34.9±2.3 ^a	19.2±3.8 ^d	30.9±1.30 ^c
K _e	11.3±1.10 ^d	29.6±4.20 ^f	11.8±0.6 ^d	39.0±1.6 ^e	11.8±0.8 ^d	35.9±3.70 ^e
Na _e +K _e	250.4±23.8	239.6±16.7	236.2±9.4 ^a	229.3±6.9 ^b	287.7±20.3	279.0±13.2
GSH	19.7±6.6	19.7±3.40	30.4±4.9	30.1±2.7	25.3±10.9	25.0±3.60

¹PCV defined as %, Na_{wb}, Na_p, Na_e, K_{wb}, K_p, K_e and Na_e + K_e defined as mmol L⁻¹; GSH as mg dL⁻¹; ^{a-f}Row values with different superscripts differ (p<0.05; p<0.01, respectively); the comparisons are only within breed

type of potassium for Saanen, Maltese and Turkish Hair goats were significant for K_e and Na_e concentrations (p<0.05). Glutathione (GSH) displayed polymorphic structure for the Saanen, Maltese and Turkish hair goat breeds. The phenotypic frequencies of glutathione types were found 80 and 59% for low GSH in erythrocyte for S and TH goats, respectively. On the contrary, the phenotypic frequencies of glutathione types were calculated 35% for low GSH and 65% for high GSH in erythrocyte for M goat. In addition, the allele gene frequencies of GSH^h-GSH^H loci were calculated as 0.89-0.11; 0.59-0.41 and 0.76-0.24 for S, M and TH goats, respectively. The mean of erythrocyte glutathione was determined 13.5-13.0 mg dL⁻¹ for low and 44.6-39.5 mg dL⁻¹ for high GSH types in erythrocyte for S and M breeds, respectively. But these values were found 8.9 mg dL⁻¹ for low and 48.5 mg dL⁻¹ for high GSH types in erythrocyte for TH goat and the differences between low and high glutathione types were statistically important for all breeds in this study (p<0.01).

Meanwhile, the phenotypic frequencies of potassium types were detected as 36 and 24% for LK; 64 and 76% for HK in S and TH goats, respectively. Also, the phenotypic frequencies of potassium types were found 27% for LK and 73% for HK in M goat. In addition, the allele gene frequencies of K^H-K^L loci were calculated as 0.80-0.20; 0.85-0.15 and 0.87-0.13 for S, M and TH goats, respectively. Finally, differences between the observed and expected frequencies of potassium and glutathione types were non-significant, thus these three populations were in Hardy-Weinberg equilibrium.

The mean of erythrocyte potassium (K_e) was determined 11.3-11.7 mmol L⁻¹ for LK and 29.6-39.0 mmol L⁻¹ for HK types in S and M breeds, respectively. Also, these values were calculated 11.7 mmol L⁻¹ for LK and 35.9 mmol L⁻¹ for HK types for TH goat and the differences between low and high potassium types were statistically important for

Table 2: Least square means of blood parameters (PCV, Na_{wb}, Na_p, Na_e, K_{wb}, K_p, K_e, Na_e+K_e and GSH) based on breeds

Blood parameters	Breed			
	Turkish hair	Maltese	Saanen	SE ¹
PCV	21.82 ^a	21.51 ^a	22.38 ^a	0.78
Na _{wb}	180.37 ^a	165.24 ^b	170.20 ^b	4.78
Na _p	163.78 ^a	158.42 ^a	157.90 ^a	5.24
Na _e	239.11 ^a	190.64 ^b	214.05 ^{ab}	18.97
K _{wb}	28.94 ^a	34.63 ^b	24.18 ^a	2.57
K _p	25.65 ^a	34.98 ^b	22.96 ^a	2.98
K _e	29.31 ^a	34.19 ^b	28.28 ^a	5.35
Na _e +K _e	279.24 ^a	224.83 ^b	242.33 ^{ab}	19.54
GSH	24.78 ^a	29.56 ^b	20.78 ^a	4.98

¹Pooled standard error based on most conservative number in a breed group; ^{a,b}Row values with different superscripts differ (p<0.05)

all breeds in this study (p<0.01). Furthermore, the mean erythrocyte sodium concentration (Na_e) for the LK and HK types of the S, M and TH goats were found 239-210 mmol L⁻¹; 214.4-190.3 mmol L⁻¹ and 275.9-236.5 mmol L⁻¹; respectively. Hematocrit (%) values (PCV) of blood were also investigated based on erythrocyte potassium type as LK and HK types of the S, M and TH goat breeds, respectively. Hematocrit values were calculated 23, 22 and 23% for both potassium types in the S, M and TH goat breeds, respectively. However, the observed differences of hematocrit values were non-significant for these breeds.

Table 2 shows least square means for PCV, Na_{wb}, Na_p, Na_e, K_{wb}, K_p, K_e, Na_e+K_e and GSH depending on breed differences. Differences between the breeds for PCV were non-significant. In terms of Na_{wb}, Turkish Hair goats had the highest amount followed by Saanen and Maltese goats. The Hair goat also was significantly different than the other two breeds while Maltese and Saanen goats did not differ (p>0.05). Differences among the breeds for Na_p were non-significant, though hair goat had the highest amount, just as in Na_{wb}. In addition, Turkish hair goat had the highest amount for Na_e followed by Saanen and Maltese. In Table 2, it was also showed that Maltese goats had the highest amount of K_{wb} followed by Turkish

hair and Saanen. Differences between the breeds for K_{wb} mmol L⁻¹ were significant for those between Maltese and the others. The same significance structure was valid for K_p and the Maltese goats had the highest amount of K_p . For the K_e mmol L⁻¹ values, similarly Maltese breed had the highest amount followed by the Turkish hair goat and the Saanen goat breeds and the differences were important statistically between Maltese and the other breeds. However, Turkish hair goats had the highest Na_e+K_e followed by Saanen and Maltese goats and the differences between TH and M were significant while no significant between TH and S and M and S breeds. Moreover, the differences among the breeds were non-significant for GSH, Maltese having the highest concentration, followed by Turkish hair and Saanen goats.

Correlation coefficients among various blood parameters for Saanen, Maltese and Turkish hair goats are showed in Table 3. PCV values were non-significantly correlated with different blood parameters when the Saanen goat blood was considered. On the other hand, the correlation between in erythrocyte Na_e and K_e was $R = -0.34$ which was statistically significant ($p < 0.05$). But PCV values were non-significantly correlated with blood parameters for the Maltese goat. The correlation between Na_e and K_e was $R = -0.32$ ($p < 0.05$). For the Turkish hair goat, PCV values were significantly correlated with Na_e and Na_e+K_e as $R = -0.36$ and -0.39 , respectively. Similarly,

the correlation between Na_e and K_e were observed as $R = -0.26$ ($p < 0.05$). Furthermore, the frequency of parity and type of birth based on erythrocyte potassium and glutathione types of all breeds were shown in Table 4. Even if the differences among the breeds for parity and the birth type were all non-significant, twinning rate in Saanen goats was higher than the other breeds. However, Turkish hair goats mostly had the single birth kids.

Conversely, Table 5 display the results of Multivariate Analysis of Variance (MANOVA) test that measured overall effects of the independent variables of breed, parity and birth type on the dependent variables of PCV, Na_{wb} , Na_p , Na_e , K_{wb} , K_p , K_e , Na_e+K_e and GSH. The only significant difference in the analysis was observed among the breeds. This is in agreement with the univariate statistics and indicates that overall, three different breeds studied were significantly different for blood parameters while parity and birth type differences were small enough to be considered non-significant.

In several studies in literature, some of the breeds were compared with each other for erythrocyte potassium and glutathione polymorphism to detect the differences among the breeds and also evaluate the gene frequency changes over time within the same breed. For example, Turkyilmaz (2003) showed the significant differences between exotic (Saanen) and Turkish native breeds (Turkish hair goat) for the potassium as well as glutathione concentrations in erythrocyte. In the present study, the potassium concentration in erythrocyte significantly different between Maltese and Turkish hair goats; also between Maltese and Saanen however, these values were non-significantly different between the Saanen and Turkish hair goats. Similarly, there was not any important relationship among the all breeds in terms of GSH concentration.

The polymorphisms on these blood parameters have also been investigated in various studies. In one study, Garcia-Casas *et al.* (1992) researched with 148 goats on Canary islands and reported that levels of erythrocyte sodium, potassium and GSH were 73.4 and 30.2 mEq L⁻¹

Table 3: Correlation coefficients among various blood parameters in Saanen, Maltese and Turkish hair goats

Blood parameters	Saanen	Maltese	Turkish hair goat
PCV- Na_{wb}	0.16	-0.17	-0.36*
PCV- Na_p	0.32	-0.11	-0.05
PCV- Na_e	-0.20	-0.18	-0.36*
PCV- K_{wb}	0.26	0.17	0.07
PCV- K_p	0.21	0.19	0.15
PCV- K_e	0.25	-0.08	-0.12
PCV- Na_e+K_e	-0.15	-0.20	-0.39**
K_e-Na_e	-0.34*	-0.32*	-0.26*
$K_{wb}-Na_{wb}$	-0.25	-0.16	-0.04
K_e-GSH	-0.06	0.06	-0.19

* $p < 0.05$, ** $p < 0.01$

Table 4: The distribution of parity and type of birth based on erythrocyte potassium and glutathione types of Saanen, Maltese and Turkish hair goat breeds

Traits	Potassium											
	Saanen GSH types				Maltese GSH types				Turkish hair goat GSH types			
	LK	HK	GSH ^h	GSH ^b	LK	HK	GSH ^h	GSH ^b	LK	HK	GSH ^h	GSH ^b
Birth type (%)												
Single	33	31	50	31	71	64	50	70	83	84	90	88
Twin	67	69	50	69	29	36	50	30	17	16	10	12
Parity (%)												
1	22	28	50	26	07	20	19	12	50	24	20	37
2	22	14	50	21	28	35	32	31	08	16	20	10
3	22	08	-	10	28	09	09	18	-	35	35	20
4>	34	50	-	43	35	36	40	39	42	25	25	33

Table 5: MANOVA test criteria for the hypothesis of no overall breed, parity and birth type effects on all blood parameters

Traits	Wilks' Lambda	p-value
Breed	0.64	0.001
Parity	0.50	0.110
Birth type	0.96	0.920

67.9 mg dL⁻¹ in erythrocyte, respectively. Similarly, Tunon *et al.* (1987) confirmed the existence of polymorphism for erythrocyte potassium in certain Spanish goat breeds. Da La Haba *et al.* (1991) reported that the erythrocyte potassium concentrations were ranged between 41 and 63 mEq L⁻¹ for HK types (66.7%) and between 6.5 and 18 mEq L⁻¹ for LK type (33.3%) in Granadia goats.

The results from present study were not incoherent with these reports in literature. In the present study, the differences between the LK and HK types within breeds were also detected significant for K_e and Na_e concentrations. Similarly, Galip and Elmaci (2001) reported that the mean of K_e were 9 mmol L⁻¹ for LK type and 48.51 mmol L⁻¹ for HK types of animals in Turkish hair goats and the differences of Na_e and K_e concentrations between the LK and HK types were highly significant.

The concentrations of glutathione in erythrocyte in the present study were also similar to those reported in the literature. A study investigating Norduz goats in Van, Turkey (Ekmekci and Mert, 2009) indicated that the erythrocyte glutathione concentrations were between 15.1 and 23.3 mg dL⁻¹. In the same study, the erythrocyte potassium concentrations were between 17.8 and 22.2 mEq L⁻¹ and significant differences were observed between the commercial herds and the herds raised in the research. Igbokwe *et al.* (1998) displayed that GSH concentration was 46.5 mg dL⁻¹ in Nigeria Sahel goats. It was also reported by another study (Turkyilmaz, 2003) that GSH level was 94.8 in Saanen goats, 29.8 mg dL⁻¹ in Turkish hair goats and over 90% of Saanen and Turkish hair goats showed the low GSH type as it was reported in present study.

Potassium and glutathione in erythrocyte displayed polymorphic structures based on gene frequency for all goat breeds in this study. Evans *et al.* (1958) reported that the gene frequencies at the two loci associated with potassium types have been estimated for different British breeds and there was a considerable variation from breed to breed.

In the present study, the gene frequencies were calculated to be 0.94 for potassium types of K^H and similarly, Galip and Elmaci (2001) reported 0.97 for K^H types in Turkish Hair goats. Likewise, the *allele* gene frequencies of K^H loci were detected as 0.80, 0.85 and 0.87 for S, M and TH goats, respectively. Also the *allele* gene

frequencies of GSH^H loci were calculated as 0.89 0.59 and 0.76 for S, M and TH goats, respectively. Similarly; Turkyilmaz (2003) displayed that the gene frequencies of glutathione types (GSH^H) were determined at 0.96 in Turkish Hair goat breeds. On the contrary, GSH^H frequency was found as 0.94 for Saanen goats.

In general, hematocrit value (PCV) was observed about 22% on average for the three breeds and the observed differences were not significant among all animals used in this study. In one study, Daramola *et al.* (2005) reported 29% of PCV for West Africa Dwarf goats. Moreover, the correlation coefficients between Na_e and K_e concentrations in erythrocyte for all breeds were found statistically significant in the present study. The correlation between erythrocyte Na_e and K_e was calculated as R = -0.34, -0.32 and -0.26 for S, M and TH goat, respectively. Galip and Elmaci (2001) reported that the important association between Na_e and K_e concentrations, measured by the high correlation coefficient of -0.74 for Turkish Hair goats.

On the contrary, Igbokwe *et al.* (1998) showed non-significant correlations between GSH concentration in erythrocyte and hematocrit values, haemoglobin or anaemia for Nigerian Sahel goats. They reported that many goats in their study (75%) had low GSH concentrations in erythrocyte.

The goats with 17% PCV and an average GSH concentration of 45 mg dL⁻¹ in erythrocyte were detected as anemic. However, sixteen animals with very low erythrocyte GSH values of about 7 mg dL⁻¹ were not anemic.

CONCLUSION

In the present study, there were no anemic animals based on PCV (%) among the all breeds. As a result, Saanen, Maltese and Turkish Hair goats were used to determine genetic structures in terms of glutathione and potassium polymorphisms in red blood cells. In addition, there was some significant negative correlation coefficient between Na_e and K_e in all breeds. Besides the widely used molecular genetic techniques today, these types of studies might be encouraged to identify genetic structures of the breeds and determine the all possible relationship between blood polymorphism and various phenotypic traits especially in local livestock breeds.

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NOMENCLATURE

S	=	Saanen
M	=	Maltese
TH	=	Turkish Hair goats
HK	=	High potassium
LK	=	Low potassium
GSH	=	Glutathione level
PCV	=	Hematocrit value
Na _{wb}	=	Whole blood sodium
Na _p	=	Plasma sodium
Na _e	=	Erythrocyte sodium
K _{wb}	=	Whole blood potassium
K _p	=	Plasma potassium
K _e	=	Erythrocyte potassium
Na _e +K _e	=	Total monovalent cation concentration in erythrocyte

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